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Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study

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Summary

Purpose: To unravel the mechanism of action of neurostimulation as a treatment for seizures, functional neuroimaging tools allow minimally invasive research in humans. We performed single-photon emission computed tomography (SPECT) in patients with epilepsy, treated with vagus nerve stimulation (VNS). Changes in regional cerebral blood flow (rCBF) at the time of initial stimulation as well as after chronic treatment were correlated with long-term clinical efficacy.
Methods: In this pilot study, 27 patients (14 female and 13 male) who were treated with VNS at Ghent University Hospital for refractory epilepsy underwent a ^{99m}Tc-ECD (ethyl cystein dimer) SPECT activation study at the time the first stimulation train was administered. 12 patients underwent an additional ^{99m}Tc-ECD SPECT activation study 6 months later. Image acquisition was performed on a high-resolution triple-headed gamma camera. Significant rCBF changes were correlated with prospectively assessed clinical efficacy data.
Results: Significant rCBF changes were found in the thalamus, the hippocampus and the parahippocampal gyrus. Acute limbic hyper-perfusion and chronic thalamic hypo-perfusion correlate with positive clinical efficacy.
Conclusions: Acute and chronic electrical stimulation of the vagus nerve induces rCBF changes that can be measured by SPECT on a group-basis. The thalamus and the limbic system are thought to play a key role in the mechanism of action of VNS.

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Introduction

In view of the extensive number of patients who cannot benefit from currently available treatments for epilepsy, the evaluation of the therapeutic potential of peripheral and intracerebral neurostimulation has regained interest in the past 15 years. Identification of the nervous tissue structures to be targeted and the optimal stimulation parameters to achieve efficacy and maintain safety are the main issues to be clarified. Progress in ongoing research unravelling the complex pathophysiology of the epileptogenic network and the mechanism by which electrical stimulation may interfere, suggest promising practical applicability of different neurostimulation modalities for patients with refractory epilepsy.

Vagus nerve stimulation (VNS) is currently a routinely applied neurostimulation modality for patients with medically or surgically refractory epilepsy with over 50,000 patients treated worldwide.[1] Several open and controlled studies have established the efficacy and safety of VNS both during short and long-term follow-up.[2–5] For patients with refractory epilepsy VNS is a cost-beneficial treatment.[6]

The exact mechanism by which VNS exert its anti-epileptic effect is not fully understood.[7] Elucidation of this mechanism might be an important step forward in the identification of predictive factors for positive outcome or guidelines for optimized stimulation regimens. The vagus nerve communicates with the nucleus of the solitary tract in the brain stem. From this location, it may influence various parts of the brain as it has widespread cortical and subcortical anatomical and functional connections.[8,9] Functional imaging techniques using activation paradigms to evaluate regional cerebral blood flow (rCBF) changes are suitable for studying VNS-induced effects in humans in a minimally invasive way. Several human functional neuroimaging studies have shown the possibility of measuring rCBF changes induced by VNS and in this way identified potentially important central nervous system structures. As most studies were performed in small and heterogeneous patient populations a consensus on key structures or type of rCBF changes that are involved has not been reached. The majority of studies reporting an increase in rCBF used positron emission tomography (PET)[10,11] or more recently also functional magnetic resonance imaging (fMRI)[12–15] whereas those reporting a decrease used SPECT.[16–18]

The current pilot study aimed at describing alterations in rCBF triggered by VNS in a larger and fully documented patient group ($N = 27$). Long-term clinical follow-up was available for all included patients. Once we identified brain regions with significantly altered rCBF, we correlated these findings with clinical efficacy data obtained after a considerable follow-up period at a time when potential clinical efficacy of VNS was clear. We also explored if baseline SPECT data compared to a gender- and age-matched control group could provide predictive factors for VNS efficacy. Based on previous research and the currently available information in the literature within this context, we hypothesized involvement of the thalamus, cerebellum, parahippocampal gyrus, amygdala and hippocampus for all conducted analyses.

Patients and methods

Patient population

Twenty-seven patients (14 females, 13 males) with refractory epilepsy were included in our study. On the basis of an extensive presurgical evaluation by a multidisciplinary epilepsy team, all patients were considered unsuitable candidates for resective surgery because of non-localizing findings or localization of the epileptic focus in functional brain tissue. The presurgical evaluation protocol has been described previously.[19] All patients were treated with chronic anti-epileptic drug (AED) polytherapy. AED dosages were not changed during the first 12 months of follow-up. The individual patients characteristics are summarized in Table 1.

All patients gave informed consent based on the SPECT study protocol approved by the local ethics committee. The surgical implantation procedure of the Neurocybernetic Prosthesis System (model 100 or 101; Cyberonics, Webster, TX, USA) and the ramping-up procedure of the stimulator have been described previously.[3]

Patients were followed up at the epilepsy clinic at regular intervals and relevant clinical data on seizure frequency, seizure severity and side effects were prospectively collected using a seizure diary kept by the patient or caregiver.

Activation paradigm (Fig. 1)

All patients underwent a split-dose (2×555 MBq) ^{99m}Tc-ECD SPECT at the time of initial VNS. The technical specifications of the split-dose activation study set-up have been described earlier.[20] The first dose was injected in baseline conditions (acute baseline condition). The second dose was injected immediately following an initial 30-s stimulation train (30 Hz, 500 μs, 0.25 mA) (acute activation condition). The first 12 patients included in the study

Table 1 Patient characteristics and clinical efficacy of vagus nerve stimulation (the first 12 patients underwent SPECT after 6 months of follow-up)

Patient	Gender	Age (years)	Seizure duration (years)	Follow-up (months)	Seizure type	CPS ± SG/ month before VNS	% Reduction in seizure frequency at maximum follow-up	AEDs before VNS	AEDs at maximum follow-up
1	F	31	9	71	CPS ± SG	16	81	3	3
2	F	16	11	64	CPS ± SG/SPS	200	100	4	3
3	F	38	30	67	CPS ± SG	8	0	3	3
4	F	12	5	54	CPS ± SG	12	50	4	5
5	M	28	16	52	CPS	30	70	2	3
6	F	39	16	53	CPS ± SG	3	70	4	4
7	F	44	23	50	CPS ± SG	10	40	3	2
8	M	36	30	50	CPS ± SG	4	50	4	3
9	M	49	35	49	CPS	30	80	4	6
10	F	41	19	46	CPS	15	80	3	3
11	M	35	13	46	CPS + SG	4	0	3	4
12	F	26	25	45	CPS ± SG	120	66	3	3
13	F	22	16	35	CPS	10	60	3	3
14	F	26	24	38	CPS ± SG	90	90	3	3
15	M	37	23	38	CPS	30	40	3	4
16	M	47	43	30	CPS + SG	30	90	4	4
17	M	10	8	30	CPS	50	60	3	3
18	M	40	3	29	CPS ± SG	8	50	4	3
19	F	44	34	28	CPS/SPS ± SG	5	70	3	3
20	F	41	39	29	CPS	4	50	2	3
21	F	30	16	29	CPS ± SG	8	50	4	4
22	M	27	9	29	CPS	7	50	3	4
23	M	31	11	27	CPS	4	25	4	4
24	M	36	20	30	CPS	30	0	3	4
25	M	21	8	19	CPS ± SG	10	95	3	3
26	M	25	20	19	CPS ± SG	10	50	2	2
27	F	36	34	19	CPS ± SG	7	60	3	3

CPS: complex partial seizure, SG: secondary generalisation, VNS: vagus nerve stimulation, AED: anti-epileptic drug, M: male, F: female, SPS: simple partial seizure.

agreed to undergo this split-dose activation paradigm again after 6 months of stimulation. The first dose was injected with VNS parameters that had been programmed over a follow-up period of 6 months to reach optimal clinical efficacy with minimal side effects (chronic baseline condition). VNS parameters had been kept stable in the weeks preceding the SPECT study. The second dose was injected after an additional 30-s stimulation train (30 Hz, 500 μs, 0.25 mA) (chronic activation condition).

For the analysis the acute baseline and acute activation condition were compared; the chronic baseline and chronic activation condition were compared; and the acute and chronic baseline were compared.

^{99m}Tc-ethyl cysteine dimer (ECD) (Neurolite; DuPont Pharmaceuticals Ltd., Brussels, Belgium) was used to estimate rCBF. During the scanning procedure a neurologist was present on-site to clinically evaluate patients and detect possible clinical seizures.

Age- and sex-matched healthy volunteers

The baseline, pre-stimulus SPECT scans of the patients were compared with those of age- and sex-matched healthy individuals. Carefully screened

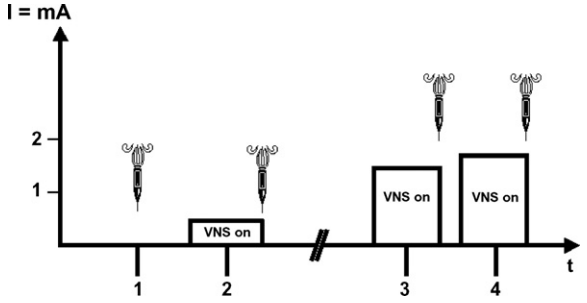

Figure 1 Acute and chronic SPECT activation paradigm. *I*: vagus nerve stimulation output intensity, mA = milliamperes, *t* = time, : ^{99m}Tc injection, 1: acute baseline condition, 2: acute stimulation condition, 3: chronic baseline condition, 4: chronic activation condition.

healthy volunteers underwent the same scanning procedure with 925 MBq ^{99m}Tc-ECD under standard circumstances. For this study, 30 healthy volunteers (16 females, 14 males; mean age 32.2 ± 8.2 years) from the GOAHEAD project were included after age and sex matching to the epilepsy patients.[21] To investigate a possible predictive value of the acute baseline condition for clinical outcome, all ^{99m}Tc-ECD perfusion scans were compared with those of the healthy volunteers and regional *z*-scores were derived. The *z*-scores were defined as the patient's ^{99m}Tc-ECD uptake expressed in standard deviations from the normal template (Brass; Nuclear Diagnostics).

Image acquisition and processing

All patient and healthy volunteer SPECT images were acquired using a GCA-9300 triple-head system (Toshiba, Tokyo, Japan) with high-resolution fan-beam collimation, uniform Sörenson attenuation correction (effective attenuation coefficient, 0.09 cm^{-1}), and triple-energy window scatter correction.[22] Reconstructed images were transferred in Interfile format onto a central image processing system (HERMES; Nuclear Diagnostics, Ltd., Stockholm, Sweden). Intra-individual scans were automatically co-registered by means of 6 rigid parameters (shift and rotate) using a count difference minimization algorithm (MultiModality; Nuclear Diagnostics, Ltd.). The average image of each patient was then anatomically standardized onto an in-house constructed database template positioned in the coordinates of Talairach and Tournoux[23] using a linear nine-parameter (shift, scale, rotation) transformation.[22] Activity changes were calculated automatically in 39 predefined volumes of interest (VOIs) including the whole-brain grey matter. In addition, the mesial temporal cortex VOI of a previously used whole-brain VOI region map was subdivided into the amygdala, hippocampus, and parahippocampal gyrus. For each individual subject and scan, the VOI activity counts were calculated per voxel and normalized onto the total number of counts in the complete VOI set of the scan.

Statistics

After validation of normal distribution of the individual values (Kolmogorov–Smirnov test) we performed a one-sample *t*-test to investigate differences in pre- and post-stimulation differences in uptake between different brain regions. All correlations were investigated using Pearson's coefficient. Statistics were calculated with SPSS (v10.0 for Windows, SPSS Inc., Heverlee, Belgium).

Results

The mean patient age at the time of implantation was 32 years (range: 10–49, S.D. = 10.2); the mean duration of epilepsy was 20 years (range: 3–43 years, S.D. = 10.9).

Clinical efficacy

The mean number of AED was 3 (range: 1–4) and this number remained unchanged at maximal follow-up.

The average follow-up period for the whole patient group was 42 months (range: 19–71 months; S.D. = 14.7). The number of reported seizures pre-VNS ranged from 3 to 200 with a mean of 28 seizures per month. At maximum follow-up period there was a mean reduction of seizure frequency of 57% (range: 0–100%; S.D. = 27.3). For the total group, 14 patients became responders and showed a >50% reduction in seizure frequency. In nine patients, VNS resulted in a moderate seizure reduction (30–50%), while four patients showed only minor or no effect of VNS (reduction <30%).

Acute baseline condition versus acute stimulation condition (Table 2)

A significant decrease in rCBF was found in the right and left parahippocampal gyrus ($p < 0.001$; $p = 0.026$), the left thalamus ($p = 0.035$) and the right hippocampus ($p = 0.016$). These rCBF changes reached a higher lever of significance when non-responders (<30% seizure frequency reduction, $n = 4$) were excluded from the analysis: right and left parahippocampal gyrus ($p < 0.001$; $p = 0.017$), left thalamus ($p = 0.019$), right hippocampus ($p = 0.010$).

Acute baseline condition versus chronic baseline condition

A decrease in ^{99m}Tc-ECD uptake between the acute baseline condition and the chronic condition was found in the left thalamus ($p = 0.034$). No other brain regions of hypo- or hyper-perfusion could be discovered. Results are summarized in Table 2. When excluding non-responders (<30% seizure frequency reduction, $n = 4$) in this analysis the decreased left thalamic blood flow gained significance ($p = 0.009$) and also the right thalamus now showed a significant decrease in rCBF ($p = 0.028$).

Chronic baseline condition versus chronic activation condition

An additional 30-s stimulus of 0.25 mA after chronic stimulation during 6 months yielded an increased

Table 2 Ratios of different study conditions and significance in hypothesized regions

Brain region	Acute stimulation condition/ acute baseline condition (n = 27)			Chronic baseline condition/ acute baseline condition (n = 12)			Chronic activation condition/ chronic baseline condition (n = 12)		
	Mean	t	p (2-tailed)	Mean	t	p (2-tailed)	Mean	t	p (2-tailed)
Left cerebellum	1.008	1.189	NS	1.015	0.746	NS	0.978	−1.428	NS
Left thalamus	0.977	−2.230	0.035	0.950	−2.417	0.034	1.035	3.180	0.011
Left amygdala	0.994	−0.230	NS	1.036	0.954	NS	1.010	0.461	NS
Left gyrus parahippocampus	0.974	−2.369	0.026	1.023	0.887	NS	0.993	−0.462	NS
Left hippocampus	1.000	0.005	NS	1.005	0.178	NS	1.017	0.794	NS
Right cerebellum	1.012	1.189	NS	1.012	0.874	NS	0.963	−1.980	NS
Right thalamus	1.000	0.033	NS	0.969	−1.656	NS	1.0127	1.192	NS
Right amygdala	1.016	0.789	NS	1.012	0.257	NS	1.020	0.786	NS
Right gyrus parahippocampus	0.972	−4.012	<0.001	0.989	−0.454	NS	0.994	−0.575	NS
Right hippocampus	0.971	−2.567	0.016	0.980	−0.609	NS	0.870	−1.321	NS

rCBF in the left thalamus (p = 0.011). No other regions among the hypothesized regions of involvement of altered rCBF could be established. In addition no changes in rCBF could be found for the other brain regions, after Bonferroni correction for multiple comparisons. Results are also given in Table 2.

Baseline SPECT versus healthy volunteers

When considering the regional z-scores of the whole group of patients, there were a number of pre-stimulus differences for the patients versus age- and gender-matched normals. Comparing the previously hypothesized brain regions in the whole patient group, relatively hyperperfused areas were present in the left parahippocampal gyrus (p = 0.036), left amygdala (p = 0.009), and left hippocampus (p = 0.035). Among the regions not previously implied in the mechanism of action of VNS, after Bonferroni correction, a relative hypoperfusion was present in the right caudate head (p_{corr} = 0.0016).

Correlations between rCBF changes and long-term clinical efficacy

When correlating the rCBF changes in the acute stimulation condition with the absolute percentage of seizure reduction, a significant correlation was found with the left medial temporal inferior region (ρ = −0,459, p_{uncorr} = 0.016). Fig. 2 demonstrates that an increased left temporal medial inferior blood flow is positively correlated with an increasing efficacy.

The chronic activation condition did not produce rCBF alterations correlating with clinical efficacy.

Correlation of rCBF changes between the chronic baseline condition and the clinical efficacy generated a positive correlation between a decreasing left thalamic rCBF and an increasing clinical efficacy (ρ = 0.615, p_{uncorr} = 0.033) (Fig. 3). No other correlations could be established.

Correlation of the pre-stimulus z-scores with the clinical outcome percentage produced no significant result.

Discussion

Despite the growing number of patients treated with VNS and the successful exploration of novel and promising indications within and even outside the neurological field, the precise mechanism of action of VNS in influencing the brain in a therapeutically efficacious manner, remains to be elucidated. Treat-

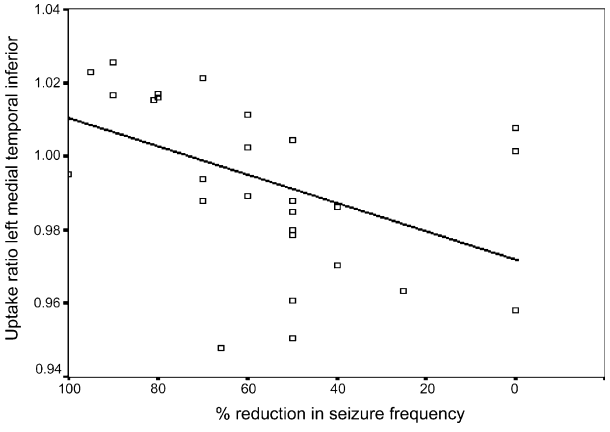

Figure 2 Positive correlation between increased left temporal medial rCBF and increasing clinical efficacy (acute stimulation condition correlated with outcome).

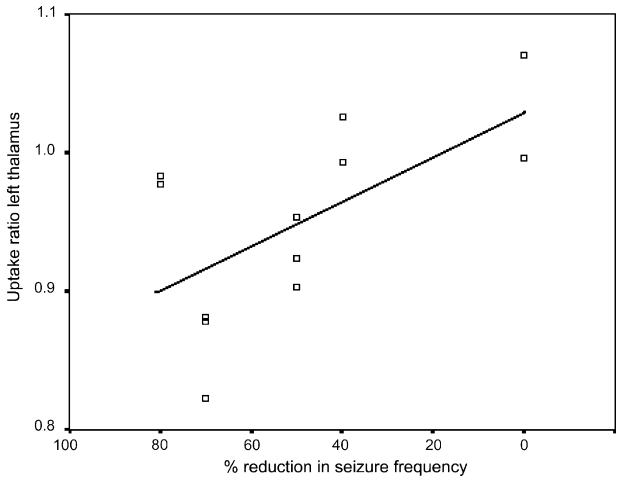

Figure 3 Positive correlation of a decreasing left thalamic rCBF and increasing clinical efficacy (chronic baseline condition correlated with outcome).

ment for epilepsy as well as other pathological conditions is mainly based on evidence-based information rather than on rational deductions from basic research in the pathophysiology or the mode of action of neurostimulation because many of these issues currently remain unclarified. This is also the case for many other medical treatments especially for neurological conditions that arise from a complex physiological substrate like the human nervous system.

In the past 10 years interesting findings have arisen from different types of research. However, these findings have so far not been useful for practical application in the sense that predictive factors for clinical response or practical guidelines for optimising stimulation parameters have been identified. Functional imaging techniques such as PET, SPECT and more recently fMRI allow to non-invasively measure neuronal activity as reflected by changing rCBF.[24] Based on the established clinical efficacy of VNS as a treatment for epileptic seizures, a condition of cortical origin, it is supposed that electrical stimulation of the vagus nerve can acutely and/or chronically change neuronal activity within the brain. Identification of the localization(s) and nature of these supposed changes may be investigated with the mentioned techniques. As VNS in a clinical setting consists of an implanted programmable biomedical system that can be turned off and on, activation studies are suitable to evaluate intracerebral functioning in relation to electrical stimulation at a certain distance. Moreover, the correlation of VNS-induced rCBF changes with VNS-induced reduction in seizure frequency is a powerful experimental design that can be performed in humans to study the mechanism of action and potentially identify predictive factors for positive clinical outcome.

In the past, different modalities of functional imaging have been used in VNS research, some of them in small patient groups at a time when VNS was still an emerging novel treatment. There are limited numbers of studies in animals.[25] Interesting results firstly arose from a study by Henry et al.[11] who examined the acute effects of VNS using PET. Correlation of acute VNS-induced rCBF alterations and chronic therapeutic responses showed that bilaterally increased thalamic rCBF correlated with a decreased seizure frequency in responders after 3 months of treatment.[26] Several other studies, using PET, SPECT and recently fMRI examined rCBF alterations after chronic VNS treatment leading to further identification of potential key structures involved in the mechanism of action. However no uniform functional pathway addressed by VNS to exert its effect from the cervical part of the vagus nerve to the brain cortex was outlined.

In the current study 27 patients were included with a mean follow-up period of 42 months. In contrast to many other anti-epileptic treatments such as anti-epileptic drugs, it is known from long-term clinical studies with VNS that efficacy increases over a time period of several months. Meaningful correlations of functional imaging results with clinical efficacy demand prospectively assessed long-term clinical follow-up.

In the acute stimulation condition we found a decreased rCBF in left thalamus, right hippocampus and bilateral parahippocampal gyri. Because we used a more detailed region map for the medial temporal cortex, we were able to identify temporal lobe substructures with altered rCBF. In previously published studies from our group using the same protocol in a smaller patient population, we described a contralateral hypo-perfusion in the parahippocampal gyrus.[27] Why effects were predominantly contralateral remains unclear and we assumed a possible role of the heterogeneous patient group included in our study. In the current study we demonstrated a bilateral involvement of the parahippocampal region. Decreased rCBF in the hippocampal region has been described previously.[11,16,18] Medial temporal lobe structures displaying VNS-induced rCBF changes are known to be involved early in the propagation of abnormal neuronal activity through the epileptic network underlying complex partial seizures. A decreased rCBF may reflect a lowered state of activity in these cortical areas preventing the propagation of epileptic activity. The same reasoning holds true for the thalamus as increasing evidence is found that the thalamus is part of the epileptic network not only in generalized seizures but also in partial seizures.[28,29] The fact that rCBF changes are induced in crucial

cortical areas even following a single short-lasting and low-output stimulation train may explain the acute abortive effect of VNS. This effect was shown both in animal models for epilepsy and in a clinical setting.[30,31] A substantial number of patients treated with VNS successfully use the magnet feature of the device. This magnet allows to manually administer an additional stimulation train in case of an aura or an arising seizure in order to interrupt early epileptic activity.

The chronic baseline condition demonstrated a decreased tracer uptake in the left thalamus confirming our own earlier hypothesis that VNS induces a chronic inhibitory state in a key structure for seizure spread resulting in higher thresholds for propagating epileptic activity.

The finding of an increased rCBF in the thalamus in the chronic activation condition might be a reflection of the fact that chronic VNS provokes sustained changes in the thalamus that lead to different reactions when an acute stimulus is administered. This situation may be due to local adaptation effects that alter synaptic neurotransmission.

When correlations with clinical efficacy were investigated, the pre-VNS baseline scans did not identify any predictive value of SPECT for future clinical outcome in patients who are considered for VNS treatment. Patients who are currently treated with VNS result from an exclusion process during the presurgical evaluation process. This yields a patient population group with many unlocalized or multifocal types of epilepsy which may partly account for this finding.

In the acute stimulation condition, the correlation with positive clinical outcome involves rCBF changes in limbic structures which is in line with our reasoning about VNS interfering with the epileptic network as described by Bertram and colleagues.[28,29] They observed a monosynaptic excitatory input from the thalamus to the limbic structures of the medial temporal lobe. Together with known connections to cortical and subcortical structures, the thalamus may exhibit a powerful modulating role in the synchronization of this neuronal circuit. Vagus nerve stimulation may influence this neuronal circuitry via inhibition of the thalamus.

The finding of specific changes in rCBF following an initial single stimulation train may be useful to identify responders before patients are implanted with a permanent device. This would imply the application of SPECT in patients in whom the vagus nerve is transcutaneously stimulated, e.g. with the use of transcutaneous electrical nerve stimulation (TENS) devices.

In conclusion we can state that the findings from our study investigating VNS-induced rCBF at different relevant time periods during VNS treatment put together with other functional imaging studies using different designs point to a central role of the thalamus and medial temporal lobe structures in the mechanism of action of VNS. Further studies specifically investigating the influence of different stimulation parameters on rCBF in these structures in humans and animals may eventually provide practical guidelines and lead to improved clinical outcome. Deep brain stimulation is an emerging treatment for epilepsy and the structures identified in VNS research have already proven to be valuable targets for direct stimulation.[32,33] Performing functional imaging study designs in patients treated with VNS and DBS may be an interesting avenue to further clarify the mechanism of action of anti-epileptic treatments as well as the pathophysiology of epilepsy itself.

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